

Characterization ,Biotyping and Genotyping of *Cronobacter sakazakii* isolated from Baghdadian foods

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Abstract

The aim of this study was to characterize, biotyping and genotyping of *Cronobacter sakazakii* previously isolated from a range of foods. Some virulence factors of 6 *Cronobacter sakazakii* were tested. All the isolates (6) were capsule formation, capable of producing biofilm, Lipase, gelatinase and protease production. Most of the isolates have hemolytic activity, while none of the isolates have the ability to hydrolyze starch or produce lecithinase. The effect of some physical and biological factors on growth of *Cronobacter sakazakii* were also studied. Optimum temperature was 40°C for (3) isolates, while 37°C for (2) isolates and 30°C for (1) isolate. Optimum pH was 5 for (4) isolates, while (2) isolates gave highest growth at pH 7. An agar well diffusion method was used to assess the antibacterial activity of *Lactobacillus fermentum* against *Cronobacter sakazakii* diameter of inhibition zone was ranged between 15-25 mm. Some biochemical tests were performed in order to biotype the isolates. The 6 isolates were distributed in to two biotypes, biotype 1 and biotype 11. BOX-PCR fingerprinting technique was used to genotype *Cronobacter sakazakii* food isolates. Three isolates (50%) were belong to different genotypes (this may be due to the differences in the source of isolation), and the other three isolates (50%) were belong to genotype with closely related patterns of fingerprints (two of strains were isolated from the same type of food sample).

Key words: *Cronobacter sakazakii*, biotyping, genotyping, characterization, foods

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Introduction

Cronobacter is an emerging genus of opportunistic Gram-negative pathogens associated with potentially fatal neonatal infections, including meningitis, sepsis and necrotizing enterocolitis (NEC)(1). Historically, *Cronobacter spp.*

were thought to be a single species known as *Enterobacter sakazakii* (2). *Cronobacter* was recognized as its own genus within the family of Enterobacteriaceae, after improved identification techniques including partial 16S rDNA, hsp60 sequencing ,polyphasic analysis and culture media. *Cronobacter spp.* contains seven species including *C. sakazakii*, *C. malonaticus*, *C. turicensis*, *C. muytjensii*, *C. dublinensis*, *C. condiment* and *C. universalis* (3). Identification of virulence factors is important in understanding bacterial pathogenesis and their interactions with the host, which may also serve as novel targets in drug and vaccine development(4). Virulence factors of *Cronobacter sakazakii* are enterotoxins ,O antigen, production of proteolytic enzymes etc. Biofilm formation may enhance the resistance to sanitizers and allow along term presence in the manufacturing environment (5). *Cronobacter spp.* repeatedly have been reported as remarkably resistant to osmotic stress and dryness and moderately thermotolerant as some encapsulated *Cronobacter spp.* were still recoverable from desiccated infant formula after storage for up to 2.5 years(6).Farmer *etal.*,(7) described 15 biogroups of *E. sakazakii* based on biochemical profiles with the wild type biogroup 1 being the most common.The biotyping scheme was based on motility,Voges- Proskauer, methyl red, indole, ornithine decarboxylase, reduction of nitrate to nitrite, production of gas from D-glucose, malonate utilization and production of acid from myo-inositol and dulcitol. A new biogroup 16 was designated by Iversen *etal.*,(8).PCR-based methods for genetic typing of bacteria are relatively cheap, easy to perform and provide fast results(2days) in comparison to Pulsed-Field Gel Electrophoresis(PFGE),which is highly discriminative and considered the reference method for genetic typing of bacteria, but also expensive and time- consuming (5days). Repetitive elements -Polymerase Chain Reaction , Rep-PCR-based fingerprinting methods including the 154bp BOX elements take advantage of the presence of repetitive sequences that are interspersed throughout the genome of diverse bacterial species (9). The 154 bp BOX elements consist of differentially conserved subunits (10). Only the boxA-like subunit sequences appear highly conserved among diverse bacteria (11). BOX-PCR was used successfully for the first time in typing *E.sakazakii* strains (12).

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Materials and Methods

-Bacterial strains

Eleven strains of *Cronobacter sakazakii* were isolated previously from different food samples in Baghdad /Iraq ,during 2014.All of the isolates were determined by chromogenic media,VITEK2 system and ultimately identified by Polymerase Chain Reaction(PCR).Six of *Cronobacter sakazakii* isolates were selected to perform this study.

-Detection of some virulence factors

The following virulence factors were performed :

-capsule formation: this test was performed according to negative staining method using india ink (13).

-Detection of biofilm production assay

It was performed according to Amaral *etal.*,(14) method with modification. This assay was performed by triplicate and the mean biofilm absorbance value was determined . The data obtained were used to classify the isolates as high producer (OD higher than 0.24) at 630nm.Weak producer between 0.125-0.250 at 630nm.Non producer less than 0.120 at 630nm ,according to Christensen *et al.*, (15).

-Detection of production of haemolysin on blood agar medium(16) ,protease on skim milk agar plates (17),amylase on starch agar medium(13) ,lipase,lecithinase on egg yolk agar (18) and gelatinase on gelatin medium(17) were also studied.

-Effect of some physical and biological factors on growth of *Cronobacter sakazakii*.

-Growth at different temperatures and pH degrees .

Each of isolate was activated by sub cultured in brain heart infusion broth(Hi-Media,India) at 37°C for 24h.Then to determine the optimal temperature and the optimal pH level for enhancing the growth of bacteria,10⁹ CFU/ml of each sample was recultured in nutrient broth(Biolife,Italy) and incubated at different temperatures(30,37,40,45and 50°C)for 24h.Optical densities were measured at 600nm.The same conditions were used to determine the optimal pH level for enhancing the growth of bacteria .Different pH levels(3,4,5,7 and 9)were examined. Each sample was processed five times in this manner.

- Effect of probiotic bacteria *Lactobacillus fermentum* on growth of *C. sakazakii*

An agar well diffusion method(19) was used to determine the antimicrobial activity of probiotics on growth of *C. sakazakii* isolates as follows: MRS broth (Hi-Media,India) was inoculated with lactic acid

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bacteria *Lactobacillus fermentum* and incubated at 37°C for 24h under anaerobic condition (to activate probiotic bacteria), 0.1ml the activated bacterial suspension was used to inoculate MRS broth and incubated at 37°C for 48h in shaker incubator , then centrifugation at 1000 rpm for 10min was done. Muller Hinton agar plates (Scharlau , Spain) were inoculated with 100µl of tested bacteria by spreading method after the turbidity was adjusted to 0.5 McFarland standard,after plates drying ,wells were made with sterile cork borer, and wells were loaded with 100µl of *Lactobacillus* culture filtrate supernatant. Plates were incubated at 37°C for 24h. Inhibition zone was measured in millimeter (mm) using a ruler.

-Biotyping of *C. sakazakii* isolates

According to Farmer *etal.*,(7) the following biochemical tests were used for biotyping of 6 *C.sakazakii* isolates,the defining tests are motility,Voges-Proskauer, methyl red, indole, ornithine decarboxylase, reduction of nitrate to nitrite, production of gas from D-glucose, malonate utilization and production of acid from myo-inositol and dulcitol.

-Genotyping of *C. sakazakii* isolates

-Chromosomal DNA extraction

DNA was isolated by using wizard Genomic DNA Purification kit, (Promega ,USA).

-BOX-PCR

Rep – PCR typing using BOX-AIR primer sequence

5- CTACGGCAAGGCGACGCTGACG -3 (20)was carried out for genotyping *C. sakazakii* isolates as follows :

PCR mixture was set up in a total volume of 10µl included 5µl of PCR green master mix(Promega,USA) , 2µl of (10pico mole/µl) primer and 2µl of template DNA have been used. The rest volume was completed with sterile deionized distilled water(Bioneer,Korea). Negative control contained all material except template DNA, D.W. was added instead of template DNA. The program that used in the thermocycler PCR instrument(Eppendorf,Germany) as follows: initial denaturation for 2 min at 94°C, 30 amplification cycles of 30 s at 94°C, 1 min at55°C and 8 min at 72°C and a final extension of 8 min at 72°C. The PCR products were separated on 1.5% agarose gel in 1X TBE buffer (Tris-Borate-EDTA buffer). 100bp DNA ladder was used to determine the exact size of these products. Following staining with ethidium bromide and visualization by ultraviolet (UV) illumination, the gels were photographed and interpreted visually.

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Results and Discussion

Cronobacter sakazakii was isolated from different food samples and identified previously according to chromogenic media Enterobacter Sakazakii Isolation Agar(ESIA),biochemical tests(VITEK2 system) and PCR techniques.The results of virulence factors were illustrated as bellow:

The results of negative staining showed that all *Cronobacter sakazakii* isolates (6) have the ability to form capsule ,the background was colored in gray while capsules surrounding the cells were colorless,and the cells were appeared as rods gray in color.

The ability of *C. sakazakii* isolates to produce biofilm were detected by using microtiter plates . Absorbance at 630nm were represented the degree of the biofilm thickness. The results indicated that all isolates have the ability to from biofilms with different thickness degrees , the absorbance value are ranged between (0.227-0.525) as shown in table 1.

Table (1)Biofilm thickness formed by *C. sakazakii* isolates

<i>C.sakazakii</i> isolates	Mean of absorbance value
CA1	0.326
OA2	0.227
TA3	0.384
LA4	0.298
HA5	0.354
HA6	0.525

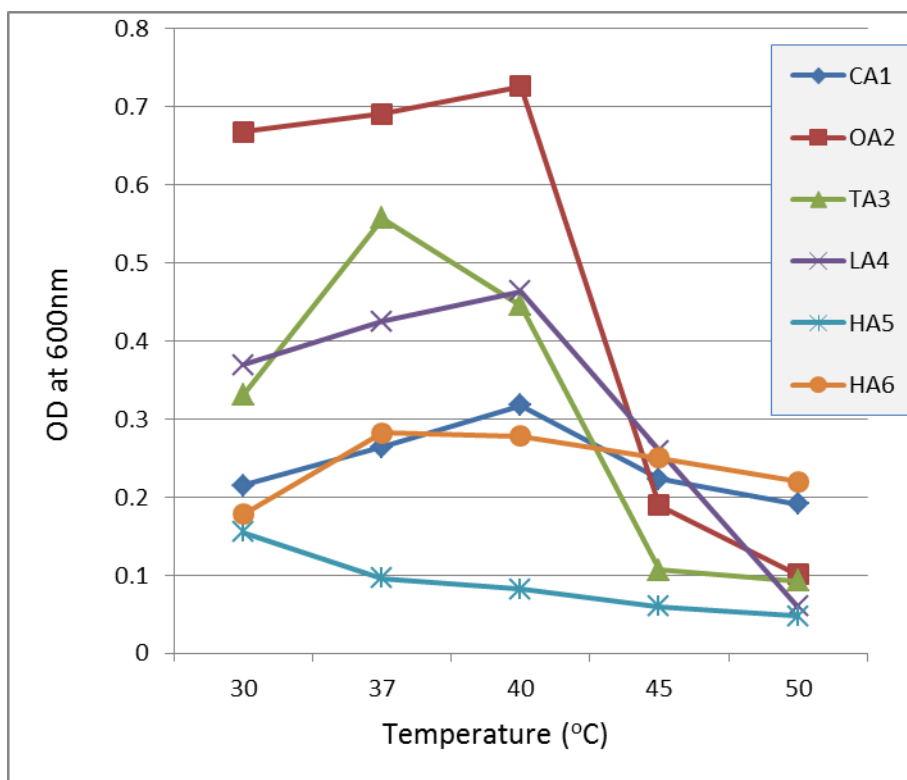
According to Christensen *etal.*,(15) 5 isolates classified as high biofilm producer including (CA1 ,TA3, LA4 , HA5 and HA6)while the isolate OA2 classified as weak biofilm producer . Fakruddin *et al.* (21) showed that *C.sakazakii* strains with higher motility had strong biofilm production ability .

– All the isolates (6) were positive for lipase, gelatinase and protease production tests. Most os the isolates have haemolytic activity,TA3 and HA6 isolates were capable of producing beta β -haemolysis around the colonies on blood agar plates,while three isolates CA1,OA2 and LA4 gave α - haemolysis and only one isolate HA5 showed no haemolytic activity (γ -haemolysis).None of the isolates have the ability to hydrolyze starch or produce lecithinase.

-Characterization of optimal growth temperatures and pH levels were shown in figure 1 and figure 2 respectively.

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Figure(1) Effect of different temperature (°C) on growth of *Cronobacter Sakazakii* isolates after incubation for 24h.

The results showed that all the isolates were able to grow at different temperature ranged between (30-40°C) and it was observed that optimal temperature was 40°C for isolates CA1,OA2 and LA4,while was 37°C for isolates TA3 and HA6, and 30°C for isolate HA5.The higher temperatures (45 and 50°C) were unsuitable for all isolates as shown in Figure (1).

Belal *et al.* (22) showed that the optimal temperature for *Cronobacter* spp. which isolated from medicinal plants and spices in Syria was 44°C,while Al-joubori , (23) showed that clinical local isolates of *C. sakazakii* can grow and survive at wide range of temperatures (10-50°C) but no growth at 4°C.

The effect of different pH levels revealed that optimal pH was 5 for four isolates (OA2,TA3, LA4, and HA6), while two isolates (CA1 , and HA5) gave the highest growth at pH(7) , as shown in figure (2).

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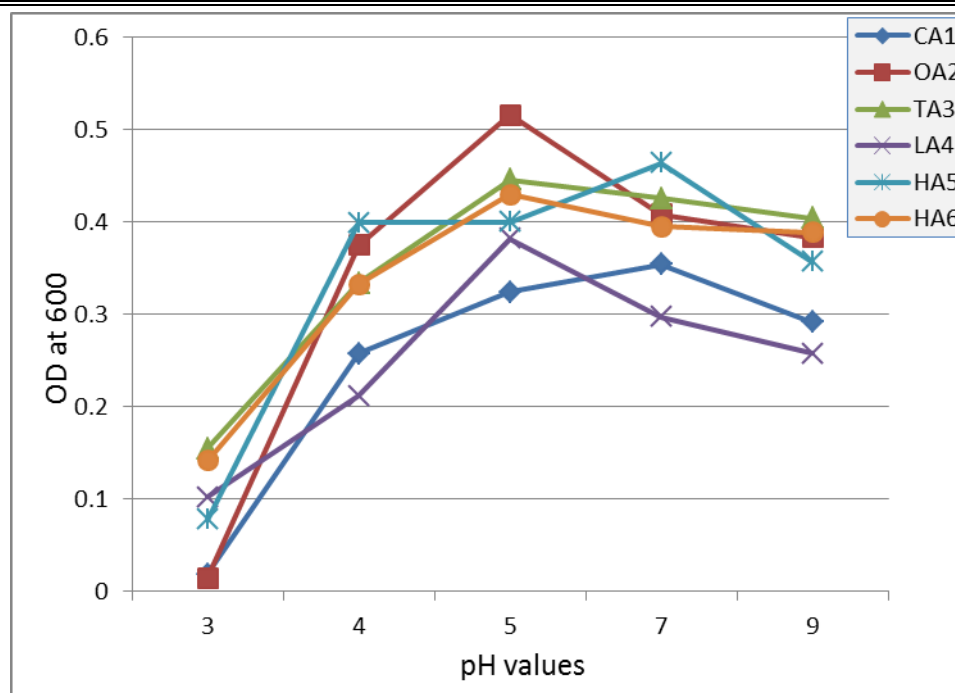


Figure (2) Effect of different pH on growth of *Cronobacter sakazakii* isolates after incubation at 37°C for 24h .

Belal *et al.*, (22) showed that the optimal pH was 5 for *Cronobacter* spp. that isolated from medicinal plants and spices in Syria.

Antibacterial activity of probiotic bacteria *Lactobacillus fermentum* against 6 food isolates of *C. sakazakii* are shown in figure (3) . The results indicated that (*L. fermentum*) Probiotic bacteria appeared antibacterial activity against all *C. sakazakii* isolates according to the inhibition zones. Diameter of inhibition zone was ranged between 15 and 25mm. Among these isolates *C. sakazakii* HA5 was the most sensitive strain which gave the maximum diameter zone of inhibition (25mm) , while LA4 isolate was the least sensitive strain which gave the minimum diameter zone of inhibition (15mm) , and the rest *C. sakazakii* isolates inhibited but in different degrees .

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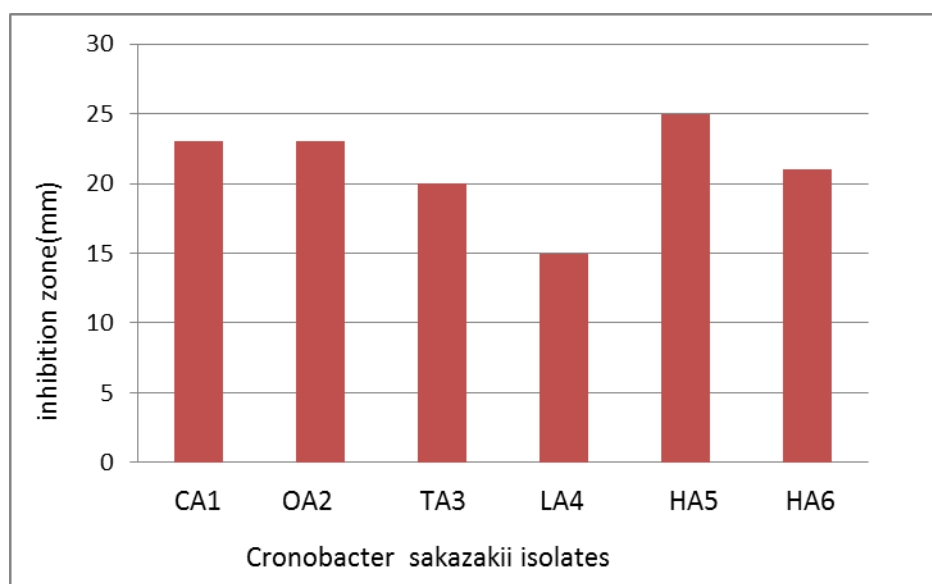


Figure (3) Antibacterial activity of *Lactobacillus fermentum* against 6 food isolates of *C. sakazakii*

Sharma and Prakash, (24) reported that *Lactobacillus fermentum* has antimicrobial activity against *C. sakazakii* incubated for various time duration .

Biotyping of *Cronobacter sakazakii* isolates-

The biochemical profiles obtained for each strain were compared to the biogroups originally described by Farmer et al.,(7). The results indicated that the isolates OA2 and TA3 were belong to Biogroup 1 or biotype 1 , while the other four isolates (CA1 , LA4 , HA5 and HA6) were belong to biogroup 11 or biotype 11 as shown in table (2).

Table (2) Biotyping of *C. sakazakii* food isolates

Biochemical profile

Biogroup	No. of isolates	VP	MR	Nit	Orn.	Mot.	Ino	Dul.	Ind.	Mal.	Gas
Biogroup 1	2	+	-	+	+	+	+	-	-	-	+
Biogroup 11	4	+	-	+	+	+	-	+	-	-	+

VP = voges proskauer , MR = methyl red , Nit = Nitrate reduction , Orn. = Ornithine decarboxylase , Mot = Motility , Ino = Production of acid from Inositol , Dul = production of acid from dulcitol , Ind = Indol Production, Mal. = Malonate utilization , and Gas = Production of gas from D–glucose

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Farmer *et al.*, (7) recognized 15 biogroups among 57 strains of *E. sakazakii* ,while Iversen *et al.*, (8) showed that 189 strains of *E. sakazakii* can be recognized to 16 biogroups and Biogroup 1 was the major group.

Biochemical evaluations of bacterial isolates are valuable for initial strain characterization and are still in widespread use . However the discriminatory power of biotyping is poor (25) and this technique has only limited ability to distinguish strains within a given species.

Genotyping of *C. sakazakii* isolates

BOX – Polymerase Chain Reaction (BOX-PCR) technique was used to genotype *C. sakazakii* local isolates. . Explanation of results was based on Woods *et al.*, (25)who illustrated that fingerprints were considered similar when all visible bands existing in each isolate had the same apparent migration distance. They were considered closely related when profiles differed by a maximum of two bands .While isolates with profiles differing by more than two bands were considered as not related . variation in intensity did not represent differences. The results showed that the fingerprints of 6 *C. sakazakii* isolates were composed of about 4 main or common bands with sizes ranging from 200 to 1500 bp as shown in figure (4) , this finding was support the identity as of same species *C. sakazakii* .

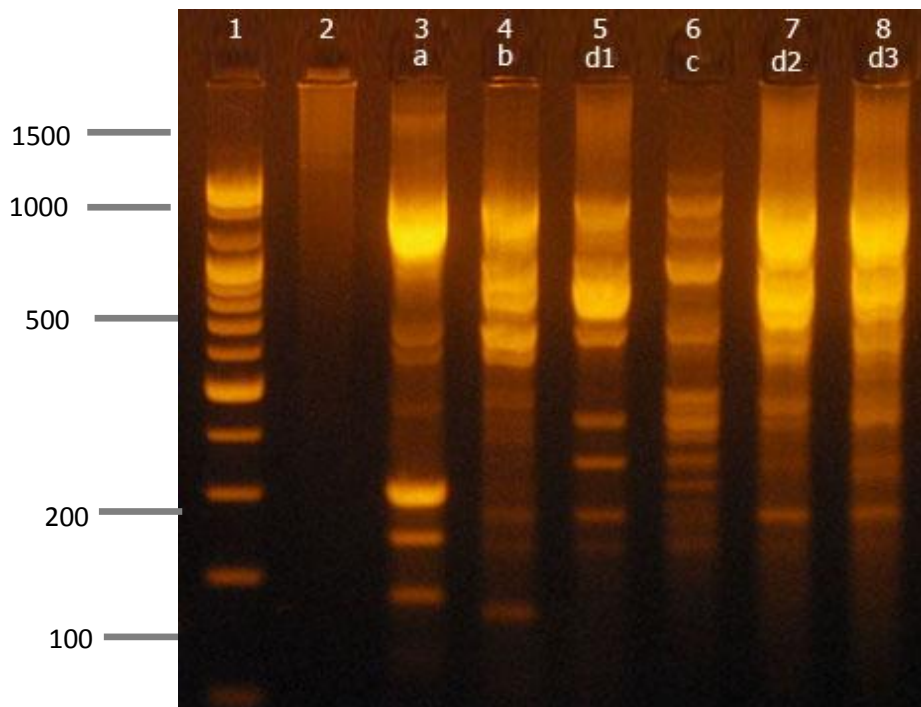


Figure (4) BOX-PCR patterns of *C.sakazakii* isolates.The PCR products were analyzed on agarose gel electrophoresis. Lane 1:100bp DNA ladder,Lane 2: negative control ,Lane 3-8: *C. sakazakii* local isolates.Genotype groups were a,b,c,d1,d2 and d3.

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These isolates also have considerable differences in the BOX fingerprints which indicates that these isolates belong to different genotypes of *C. sakazakii* .Visual analysis of the BOX fingerprints revealed a major genotype comprising (50%)of the isolates with closely related profiles (BOX – PCR types d1 , d2, and d3) this BOX – PCR types belong to three isolates TA3 , HA5 and HA6 respectively,two of which isolated from the same type of food sample(hamburger)thats indicate this genotype may be dominant in this type of food . The remaining isolates were not related to type (d1 , d2 and d3) and were distributed in 3 genotypes comprising one isolate. A high level of genetic diversity was observed among the 6 strains isolated from different food samples , the fingerprints (a, b,c,d1,d2 and d3) were obtained . Fifty percent (50%) of the isolates were not related with BOX- PCR fingerprints (a,b,c types) which belong to three isolates CA1, OA2 and LA4 respectively. A high level of genetic diversity was observed among the 6 strains isolated from different food samples , the fingerprints (a, b,c,d1,d2 and d3) were obtained . These isolates were isolated from different food samples which may also contribution to this difference in Box fingerprints (12), Proudly *et al.*,(27) showed that BOX- PCR genotyping is a very useful tool to understand the routes of contamination and distribution of *E.sakazakii* in PIF manufacturing facility. Fakruddin *et al.* (21) showed that *C.sakazakii* isolates have significant differences in the BOX fingerprint that indicates that these isolates belong to different genotypes of *C. sakazakii*.

References

1. Hunter, C.J. and J.F. Bean, 2013. Cronobacter: an emerging opportunistic pathogen associated with neonatal meningitis, sepsis and necrotizing enterocolitis. *Journal of Perinatology*, 33: 581-585.
2. Farmer, J.J., Davis B.R., Hickman-Brenner F.W., McWhorter A., Huntley- Carter M.A., Asbury M.A., Riddle C., Wathen-Grady H.G., Elias C., Fanning R., Steigerwalt A.G., O'Hara C.M., Morris G.K., Smith B.P., Brenner D.J. (1985). Biochemical identification of new species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. *Journal of Clinical Microbiology* 21, 46-76.
3. Iversen, C.; Mullane, N.; McCardell, B.; Tall, B.D.; Lahnet, A.; Fanning, S.; Stephen, R.; and Joosten H. (2008). *Cronobacter* gen. nov., a new genus to accommodate the biogroups of *Enterobacter sakazakii*, and proposal of *Cronobacter sakazakii* gen. nov., comb nov., *Cronobacter malonaticus* sp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp.nov., *Cronobacter* genomospecies 1, and of three subspecies, *Cronobacter dublinensis* subsp. *dublinensis* subsp. nov., *Cronobacter dublinensis* subsp.*lausannensis* subsp. nov. and *Cronobacter dublinensis* subsp. *lactaridi* subsp. nov. *International Journal of Systematic and Evolutionary Microbiology* 58, 1442-1447.

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4. Fakruddin M, Mazumdar RM, Chowdhury A, Mannan KSB(2013) A preliminary study on virulence factors and antimicrobial resistance in extra-intestinal pathogenic *Escherichia coli* (ExPEC) in Bangladesh. *Indian J Med Res.* 137:988–990.
5. Kim, H., Ryu, J.H. and Beuchat, L.R. (2007). Effectiveness of disinfectants in killing *Enterobacter sakazakii* in suspension, dried on the surface of stainless steel, and in a biofilm. *Applied Environmental Microbiology*, 73, 1256–1265.
6. Barron, J.C. and Forsythe, S.J. (2007). Dry stress and survival time of *Enterobacter sakazakii* and other *Enterobacteriaceae* in dehydrated powdered infant formula. *Journal of Food Protection*, 70: 2111-2117.
7. - Farmer III, J. J.; Asbury, A.; Hickman, F. W.; Brenner, D. J.; and the *Enterobacteriaceae* study group. (1980). *Enterobacter sakazakii*: a new species of "*Enterobacteriaceae*" isolated from clinical specimens. *Int J Syst Bacteriol* 30, 569- 584.
8. Iversen, C., Waddington, M., Farmer, J. J., 3rd, and Forsythe, S. J. (2006). The biochemical differentiation of *Enterobacter sakazakii* genotypes. *BMC Microbiol* 6, 94.
9. Versalovic, J., Koeuth, T. and Lupski, J.R. (1991) Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res* 19, 6823–6831.
10. Martin, B., Humbert, O., Camara, M., Guenzi, E., Walker, J., Mitchell, T., Andrew, P., Prudhomme, M., Alloing, G., and Hakenbeck, R. (1992). A highly conserved repeated DNA element located in the chromosome of *Streptococcus pneumoniae*. *Nucleic Acids Research*, Vol.20, No.13 (July 1992), pp. 3479-3483, ISSN 0305-1048
11. Versalovic, J., Schneider, M., de Bruijn, F. J. and Lupski, J.R. (1994). Genomic fingerprinting of bacteria using repetitive sequence based polymerase chain reaction. *Methods in Molecular and Cellular Biology*, Vol.5, No.2, pp. 25–40, ISSN 0898-7750.
12. Proudly I, Bougle D, Coton E, Coton M, Leclercq R, Vergnaud M. (2008a) Genotypic characterization of *Enterobacter sakazakii* isolates by PFGE, BOX-PCR and sequencing of the *fliC* gene. *J Appl Microbiol.*104:26–34.
13. Atlas, R.M.; Brown, A.E. and Parks, L.C.(1995). *Experimental Microbiology laboratory manual*. Mosby-Year Book, Inc. USA.
14. Amaral, M.M.; Coelho, L.R.; Flores, R.P.; Souza, R.R.; Silva- Carvalho, M.C.; Teixeira, L.A.; Ferreira-Carvalho, B.T.; and Figueiredo, A.M.S.(2005). The Predominant Variant of the Brazilian epidemic clonal complex of methicillin-resistant *Staphylococcus aureus* has an enhanced ability to produce biofilm and to adhere to invade airway epithelial cells. *J. Infect. Dis.* 192:801-810.
15. Christensen, G.D.; Simpson, W.A.; Baddour, L.M.; Brett, F.F.; and Metton, D.M. (1985) Adherence of Coagulase-Negative Staphylococci to plastic Tissue culture plates: A Quantitative model for the Adherence of Staphylococci to Medical Device.
16. Atlas, R.M.(2005). *Handbook of media for environmental microbiology*. 2nd ed. Tylor and Francis Group, LIC. USA.
17. Collee, J. G.; Miles, R. S. and Watt, B. (1996). Test for the identification of bacteria. In: *Practical Medical Microbiology*. 14th ed. (Collee. J. G.; Fraser, A. G.; Marmion, B. P. and Simmons, A. (eds)). Churchill Livingstone New York.
18. Edwards, P.R.; Ewing, W.H.(1972). *Identification of Enterobacteriaceae* . 3rd ed. Minneapolis, Minnesota, Burgess.
19. Barefoot, S.F.; and Klaenhammer, T.R.(1984). Detection and Activity of Lactacin B, a Bacteriocin produced by *Lactobacillus acidophilus* . *Appl. Environ. Microbiol.* 45:1808-1815.
20. Koeuth, T., Versalovic, J. and Lupski, J.R. (1995) Differential subsequence conservation of interspersed repetitive *Streptococcus pneumoniae* BOX elements in diverse bacteria. *Genome Res* 5, 408–418.

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21. Fakruddin,M.;Rahaman,M.;Ahmed,M.;andMahfuzul Hoque ,M.(2014).Stress tolerant virulent strains of Cronobacter sakazakii from food.Biological Research.47:63. doi:10.1186/0717-6287-47-63.
22. Belal,M.;Al-Mariri,A.;Hallab,L.;and Hamad,I.(2013).Detection of Cronobacter spp.(formerly Enterobacter sakazakii)from medicinal plants and spices in Syria.J.Infect.Dev.Cttries.7(2):082-089.
23. Al-joubori,Y.S.H.(2014).Isolation and Identification of Cronobacter sakazakii and Enterobacter spp.in meningitis,necrotizing enterocolitis and infant food in Baghdad. M.SC.Thesis College of science for women Baghdad University.
24. Sharma,G.;and Prakash,A.(2013).Susceptibility of Cronobacter sakazakii to plant products,antibiotics and lactic acid bacteria. International Journal of Nutrition, Pharmacology, Neurological Diseases.3:263-268.
25. Bingen, E.H.; Denamur,E.;and Elion, J.(1994). Use of ribotyping in epidemiological surveillance of nosocomial outbreaks. Clin Microbiol Rev . 7: 3 11-327.
26. Woods, C.R., Jr, Versalovic, J., Koeth, T. and Lupski, J.R. (1992) Analysis of relationships among isolates of Citrobacter diversus by using DNA fingerprints generated by repetitive sequence-based primers in the polymerase chain reaction. J Clin Microbiol 30, 2921–2929.
27. Proudly I, Bougle D, Leclercq R, and Vergnaud M.(2008b) Tracing of Enterobacter sakazakii isolates in infant milk formula processing by BOX-PCR genotyping. J Appl Microbiol.105:550–558.

توصيف , تنميط حيوي وجيني ل *Cronobacter sakazakii* المعزولة من الاغذية البغدادية

علياء رزوقي اللامي, سناء برهان عبد الجليل, عبد الكريم القرزاز

الخلاصة

هدف هذه الدراسة هو لتوصيف , تنميط حيوي وتنميط جيني ل *Cronobacter sakazakii* التي عزلت سابقا من مدى من الاغذية . اختبرت بعض عوامل الضراوة لست عزلات من *Cronobacter sakazakii* . كل العزلات كانت مكونة للكبسولة , ولها القابلية على انتاج الغشاء الحيوي ومنتجة لكل من انزيمات gelatinase, lipase , وال protease . معظم العزلات لها فعالية تحلل الدم , بينما لاتمتلك أي عزلة القابلية لتحلل النشا او لانتاج Lecithinase.

درس تاثير بعض العوامل الفيزيائية والحيوية في نمو *Cronobacter sakazakii* . درجة الحرارة المثلى هي 40°C لثلاث عزلات , بينما كانت 37°C لعزلتين و 30°C لعزلة واحدة.

دالة الحموضة pH المثلى هي 5 لاربع عزلات , بينما عزلتين اعطت اعلى نمو عند pH 7 . استخدمت طريقة الانتشار بحفر الاكار لتقييم الفعالية ضد بكتيرية ليكتريا *Lactobacillus fermentum* ضد *Cronobacter sakazakii* , يتراوح قطر منطقة التثبيط بين 15-25 ملليمتر .

لتنميط العزلات حيويا انجزت بعض الاختبارات الكيمياء الحيوية , وزعت العزلات الستة الى نمطين حيويين هما biotype1 و biotype 11 .

استخدمت تقنية ال BOX-PCR لتنميط عزلات الاغذية من *Cronobacter sakazakii* اظهرت النتائج ان ثلاث عزلات (50%) تعود الى ثلاث انماط جينية مختلفة وهذا قد يعود الى الاختلاف في مصدر العزل , بينما العزلات الثلاثة الاخرى (50%) تعود الى نمط جيني وباشكال او انماط مترابطة ومتقاربة ((اثنان من العزلات عزلت من نفس نوع العينة الغذائية)).